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Evaluation of an on-farm method to assess colostrum IgG content in sows

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Short title: Refractometer to estimate swine colostrum IgG

Abstract

The objective of this work was to investigate the evaluation of swine colostrum immunoglobulin G (IgG) concentration using the Brix refractometer. Colostrum samples were collected across all teats, from 124 sows of mixed parities. According to sampling time, three categories were created: samples available from nine hours before the onset of parturition until the first piglet was born were classified as before farrowing; samples collected after the first birth until four hours later were classified as during farrowing; and finally samples collected from this point until 14 hours after parturition, were classified as after farrowing. Samples were drawn and divided into three portions; one was immediately analyzed, a second was refrigerated and the third was frozen at -20 °C. Fresh and refrigerated colostrum samples were analyzed at the farm with a Brix refractometer. IgG content of frozen samples was analyzed using a Brix refractometer, with a subset of 42 samples also tested with a

commercially available Radial Immune Diffusion (RID) kit. The Brix percentage ranged from 18.3% to 33.2%. Brix percentage repeatability, assessed by the intraclass correlation coefficient (ICC), was very strong (fresh ICC = 0.98, refrigerated ICC = 0.88, and frozen ICC = 0.99). One-way repeated-measures ANOVA showed that storage temperature did not affect BRIX percentage of colostrum IgG ($P > 0.05$). ANOVA results show a significant effect of sampling time on colostrum immunoglobulin concentration, measured with both Brix and RID (Brix: $P < 0.003$; RID: $P < 0.05$). Immunoglobulin G concentration measured by RID ranged from 13.27 to 35.08 mg/mL. Pearson correlation coefficient revealed that Brix percentage was positively correlated ($r = 0.56$, $P < 0.001$) with RID results (regression equation: $\text{RID} = 1.01 (\pm 0.2) \text{ Brix} - 1.94 (\pm 5.66)$; $R^2 = 0.31$). The results of this study indicate that the Brix refractometer provides a simple, fast, and inexpensive estimation of colostrum IgG in sows.

Keywords Brix refractometer, colostrum IgG, evaluation, on-farm method, swine

Implications

A major problem of the pig industry is pre-weaning mortality. Piglet performance, immediate and long term survival depend on early intake of good quality colostrum. An on-farm tool such a Brix refractometer will facilitate investigation under field conditions of colostrum IgG content. By reducing cost and time needed for assessment, large scale studies, such as those looking for a genetic contribution, will be made more feasible. Moreover a rapid evaluation tool will allow farmers to feed weak piglets with fresh good quality colostrum, improving welfare and economy.

Introduction

Early and sufficient intake of good quality colostrum is essential for the health and growth of the piglet (Quesnel *et al.*, 2012). Because colostrum yield and composition vary widely among animals, it is of fundamental importance to investigate, on a large scale, the factors affecting composition. This knowledge is needed to ensure an adequate intake of quality colostrum by piglets as quickly as possible after birth, and hence reduce pre-weaning piglet mortality and lifetime risk of disease. Moreover, early postnatal management of piglets is becoming an increasing priority (Boulot, 2009). In this regard, an on-farm method for detecting colostrum IgG concentration is of primary importance to piglet welfare, health, and farm economics. The methods that have been commonly used for the determination of IgG concentration in swine colostrum are the enzyme-linked immunosorbent assay (ELISA) and the gold standard radial immunodiffusion method (RID), both not suitable for on-farm use. In other species, evaluation of colostrum IgG in the farm is a practise used to reduce failed transfer of passive immunity. For this purpose, the most recent method used to evaluate colostrum quality in sheep (Harker, 1978), horses (Chavatte *et al.*, 1998, Cash, 1999) and dairy cattle (Morrill *et al.*, 2012, Quigley *et al.*, 2013, Bartier *et al.*, 2015) is the Brix refractometer. Brix is a rapid, accurate, and inexpensive method, based on the ability of protein to refract light as a measure of total protein in colostrum (Chavatte *et al.*, 1998). To our knowledge, such on-farm tests have not yet been validated for swine colostrum. The aim of this research was therefore to establish if the Brix refractometer could be adopted also for swine. The hypothesis tested was that refraction grade in colostrum allows a meaningful estimation of swine IgG concentration as shown in several other species.

Materials and Method

Animals

The experiment was conducted at Cockle Park Farm, Newcastle University, Newcastle upon Tyne, UK, and was approved by the Animal Welfare and Ethics Review Body at the University. Colostrum samples were collected from 12 farrowing batches between November 2012 and April 2014. In total samples were collected from 124 sows of different parities (19 sows in each of parities one, three, and four; 24 sows parity two; 18 sows parity five; 13 sows parity six; 3 sows parity seven; 5 sows parity eight; 1 sow each in parity nine, ten, eleven, and twelve. Considering that there were few sows with more than six parities, a group of 12 sows with more than six parities was created). In accordance with normal commercial farm procedures, animals were moved from the group gestation house to the farrowing unit at 110 days post-insemination, where they were kept in individual crates equipped with a feeder and drinker. Ambient temperature averaged 21 °C. Sows were allowed to farrow normally at term over a 4-day period (Monday to Thursday); sows that had not farrowed within this period were then induced by injection of a prostaglandin analogue on Thursday.

Data Collection

Samples of colostrum were collected when available around parturition, without the use of oxytocin. According to sampling time, three categories were created. Devillers *et al.* (2004) defined a pre-farrowing phase when colostrum could be easily expressed manually from the teats; on average this was from 8 h before the onset of parturition. That study showed that IgG concentration was high before farrowing and

decreased rapidly thereafter. Based on this finding, samples available before the onset of parturition until the first piglet was born were classified as before farrowing; samples collected after the first birth until 4 h later were classified as during farrowing; and finally samples collected from this point until manual expression was no longer possible, were classified as after farrowing. The operator quietly approached the sow and obtained a sample by using hand pressure, exerted approximately in the centre of the mammary gland; this system seemed to aid in making the colostrum flow more freely (Fraser, 1984). A 15 mL sample of colostrum was collected by sampling from all the teats located in the upper row and, when possible without disturbing the sow, also from the teats in the lower row of the udder. The sample was collected in a sterile pot (30mL Polystyrene Universal Container, Starlab) labelled with identity of the sow, sampling time, and date. After the samples were drawn they were immediately divided into three aliquots; one was immediately analyzed (fresh colostrum at room temperature), the second was refrigerated at 4°C for 24, 48 or 72 h before being analyzed and the third sample was frozen and stored at -20°C until further analysis.

Colostrum Sample Analyses

The Brix refractometer (MA871 digital, Obione), was calibrated with distilled water before each set of analyses. A drop of well mixed whole colostrum was placed on a Brix refractometer prism and the Brix percentage (%) was recorded. Storage temperature effects were assessed on 65 colostrum subsamples, for which fresh, refrigerated, and frozen samples were analyzed in turn by Brix refractometer. Each sample was analyzed on two consecutive occasions each time to determine repeatability of results. The sampling time effect on IgG concentration was tested on

124 frozen colostrum samples, and analyzed by Brix refractometer. The correlation
between RID and Brix percentage was assessed on a subset of 42 frozen colostrum
samples. Samples selected were thawed in a warm water bath and thoroughly mixed
before RID analysis. Five microliters of mixed colostrum solution was added to each
well of a swine IgG RID test plate (Triple J Farms, Bellingham) in duplicate. Radial
immunodiffusion plates were incubated for 24 h and the diameter of the precipitin
ring was measured and compared with a standard curve created by the internal test
standards, to determine IgG concentration.

128 *Statistical Analyses*

129 Prior to statistical analysis, all data were checked for statistical outlier values. No
130 data were excluded for this reason. Descriptive statistics were performed, and data
131 are reported as arithmetic mean and standard deviation (mean \pm SD). Normality was
132 assessed by application of the Shapiro-Wilk test. Brix percentage repeatability was
133 assessed with the intra-class correlation coefficient. Data are highly considered
134 reproducible when ICC > 0.8 (Wolak *et al.*, 2012). The storage temperature and
135 length effect on colostrum IgG were analyzed using one-way repeated-measures
136 ANOVA. – Three-way ANOVA was used to investigate the effect on Brix percentage
137 of fresh samples of colostrum sampling time category (three levels), sow parity
138 number (seven levels) and farrowing induction (two levels). Where significant
139 differences were found, a Tukey test was applied as a *post-hoc* tests between
140 multiple means. The Pearson correlation coefficient was calculated to determine the
141 association between Brix and RID results, and a regression equation was produced.
142 The level of significance was taken as $P < 0.05$. The statistical software R version
143 3.0.2 (2013-09-25) was used for all tests.

Results

Descriptive statistics

Descriptive statistics of colostrum measurements are summarised in Table 1. Colostrum was collected around parturition: 31% of the samples were collected before farrowing (maximum nine h before); 41% during farrowing (from the first piglet born until four h later), and 27% after farrowing (from four h after the first piglet born, until maximum 14 h later). Only three sows did not fully expose at least one row of teats; thereby colostrum was collected from exposed teats. Twenty six percent of the animals showed only one row, 16% showed both rows but only the anterior teats of the lower row; and 54% showed completely all the mammary glands. Only 20% (N = 29) of the sows farrowed over the first four days after entry to the farrowing crates; 79% (N = 95) of the animals were then induced by injection of a prostaglandin analogue.

Brix percentage for fresh colostrum ranged from 18.3 to 33.0%, with a mean of 24.1% and SD 2.65 (Table 1). Shapiro-Wilkinson normality test displayed a normal distribution for Brix percentage of the full set of 124 samples (Shapiro- Wilkinso n P > 0.05). Radial Immune Diffusion IgG concentration of the subset of 42 samples used for comparison had a normal distribution (Shapiro- Wilkinso n P > 0.05), which ranged from 13.3 mg/mL to 35.0 mg/mL. The mean and SD of RID of the 42 samples were: 22.5 mg/mL \pm 5.43.

[Table 1 near here]

Repeatability, storage temperature and storage time effect

Repeatability of Brix percentage was assessed in a subset of 64 samples for fresh and refrigerated storage temperature and duration; due to shipping loss only 51 frozen samples were used. Brix percentage was highly reproducible for fresh (ICC = 0.98), refrigerated (ICC = 0.88), and frozen (ICC = 0.99) samples. One-way repeated measure ANOVA test results showed no significant differences ($P > 0.05$) in mean Brix percentage for different refrigeration lengths (24 h, 48 h, and 72 h). Since no significant difference was found between refrigeration lengths, only the Brix percentage values at 24 hours were used to compare the different storage temperatures. A one-way repeated measures ANOVA test was used to assess if there were differences between storage temperatures. There were no differences between fresh samples and those stored at refrigerated temperature or frozen ($P > 0.5$).

Sampling time, parity, and farrowing induction effect

ANOVA results show a significant effect of sampling time on colostrum immunoglobulin concentration, measured with both Brix (N = 124: 38 samples were collected before farrowing; 49 during farrowing; and 37 after farrowing) and RID (N = 42: 13 samples were collected before farrowing; 18 during farrowing; and 11 after farrowing). The Tukey *post hoc* tests revealed that samples collected after farrowing had lower IgG content than before and during farrowing (Brix: $F_{2,103} = 6.04$, $P < 0.003$; RID: $F_{2,32} = 2.86$, $P < 0.05$; Table 2). Figure 1 presents the result of correlation analysis between fresh and refrigerated colostrum samples for IgG concentration measured with Brix percentage, and also shows the sampling time effect on this measure. There was no effect of sow parity number on IgG concentration (Brix: $F_{6,103} = 1.00$, $P = 0.42$; RID: $F_{6,32} = 0.47$, $P = 0.82$); Furthermore, Brix percentage were not

significantly different between sows which were induced and animals that farrowed naturally (Brix: $F_{1,103} = 0.51$, $P = 0.47$; RID: $F_{1,32} = 0.31$, $P = 0.57$. Table 2).

[Table 2 near here]

[Figure 1 near here]

Relationship between Brix and RID values

Pearson correlation coefficient results showed a significant association between the Brix and RID values ($r = 0.56$; $P < 0.001$, Fig. 2). Further analysis of the data gave the following regression equation: $RID = 1.01(\pm 0.2) \text{ Brix} + 1.94 (\pm 5.66)$; ($R^2 = 0.31$, $P < 0.001$).

[Figure 2 near here]

Discussion

The Brix refractometer was evaluated as an on-farm methodology to assess colostrum IgG concentration in swine. Refractometry measures the concentration of any solution of dissolved solids, based on the degree to which the light rays are bent. Brix refractometer estimates colostrum IgG by reporting a Brix percentage (measure of refractive index), which can then be correlated with colostrum IgG concentration. Porter (1969) and Curtis (1970) quantified the immunoglobulin in sow colostrum during farrowing, they noted that 80% of the proteins in sow colostrum were immunoglobulin. Klobasa *et al.* (1987) further investigated the composition of colostrum throughout farrowing and showed that immunoglobulin concentration changed rapidly after the first piglet was born (initially 95.6 mg of IgG/mL, 21.2 mg of IgA/mL and 9.1 mg of IgM/mL) and decreased by half in the first 12 h postpartum (to

32.1 mg of IgG/mL, 10.1 mg of IgA/mL and 4.2 mg of IgM/mL). This rapid fall has also been reported by others (Klobasa *et al.*, 1987, Bland *et al.*, 2003).

The results of our current study show a significant correlation between the digital Brix instrument and the RID determination, however RID values in this study seem to be lower than previous investigations (Klobasa *et al.*, 1987, Machadoneto *et al.*, 1987, Cabrera *et al.*, 2012). These discrepancies may be due to a different sample preparation; as unfiltered whole colostrum was used in the present study in contrast to previous reports. Furthermore, the RID assay for IgG in the colostrum kit used provides standards that derive from serum IgG, which contains a different ratio of IgG₁:IgG₂; this difference can cause erroneous quantification (Gapper *et al.*, 2007). Moreover Ig concentration at parturition seems to vary from one study to another, independently of the analytical methods used. Colostrum yield and composition vary among animals depending on many factors including parity, breed, season, udder section, vaccination, number of sows per farm (Inoue, 1981a), number of stillborn piglets (Quesnel, 2011) and farrowing induction (Foisnet *et al.*, 2011). However in this study no difference was detected between Brix percentage of sows that farrowed naturally or were induced. This is likely to be because sows in the current study were only induced if they had not farrowed naturally by day 114, and therefore all farrowing occurred close to the natural gestation length for this farm. Furthermore no difference was detected between Brix percentages of sows with different parities numbers. Although this result differs from some published studies, where it was found that multiparous sows had higher IgG concentration than gilts (Inoue, 1981a; Klobasa *et al.*, 1987; Quesnel 2011; Cabrera *et al.*, 2012), it is consistent with the report of Devillers *et al.* (2004).

237 More important is that the findings of the current study are consistent with those of
238 previous authors who evaluated the relationship of results obtained using the digital
239 refractometer and standard laboratory methods on bovine colostrum ($R^2 = 0.41$,
240 Chigerwe *et al.* (2008); $R^2 = 0.53$, Biemann *et al.* (2010); $R^2 = 0.56$, Quigley *et al.*
241 (2013); $R^2 = 0.59$, Bartier *et al.* (2015)) Our results, like the previous ones on bovine
242 colostrum, did not show an extremely strong correlation between RID and Brix
243 values, as has been reported in the literature for equine colostrum ($R^2 = 0.85$;
244 Chavatte *et al.* (1998)). Biemann *et al.* (2010) suggested that the composition and
245 the volume of colostrum that is produced in mares and cows could explain these
246 differences in the results. Swine colostrum composition at farrowing contains 24–
247 30% dry matter, 15–19% total proteins, 5–7% fat, 2–3% lactose and 0.63% ash
248 (Klobasa *et al.*, 1987, Csapo *et al.*, 1996). Devillers *et al.* (2004) reported that an
249 average volume of 3.6 L (range from 1.9 to 5.3 L) of colostrum was produced by
250 sow, and IgG concentration in colostrum has been measured to average around 50
251 mg/mL (Ariza-Nieto *et al.*, 2011). Production of colostrum by cows on average was
252 11.2L, with a mean composition of fat 6.7%, total solids 27.6%, and total protein
253 14.9%, and contains 30 to 96 mg/mL of IgG (Morin *et al.*, 2001, Kehoe 2007). In
254 mares colostrum volume was 5.1L, with a much higher IgG concentration 440
255 mg/mL, and with 24.3% total solids, and 26.3% fat (Csapo *et al.*, 1995). Comparing
256 these compositions, one possible explanation for the weaker relationship between
257 Brix value and IgG content in swine and cows is that IgG represents a smaller
258 proportion of total solids. Fat and casein concentration may affect the refractometer
259 reader (Biemann *et al.*, 2010), and therefore the correlation could be influenced by
260 differences in these components of colostrum composition, which have been well

demonstrated to vary a lot between sows (Inoue, 1981b) as well as in cows (Morin *et al.*, 2001)

The effect of storage temperature on colostrum IgG assessment using the Brix refractometer was evaluated and Brix percentages were the same for samples which were fresh, refrigerated or frozen. Similar results were obtained in previous studies to validate the digital refractometer to estimate bovine and equine colostrum IgG (Chavatte *et al.*, 1998, Biemann *et al.*, 2010). Morrill *et al.* (2015) showed that there was no effect of storage temperature of cow colostrum on Brix percentages. Furthermore, they demonstrated that one freeze- thaw cycle (after 7 days) did not affect IgG content, measured by RID, and that a difference in colostrum IgG between stored samples was detected only after two freeze- thaw cycles. We also assessed the effect of length of refrigeration period to determine the consistency of measurement of IgG in colostrum refrigerated for more than 24 hours. The results suggest that the methodology is robust to procedural variations on farm.

The mean value for IgG after four hours from the onset of farrowing was significantly lower than in colostrum samples taken before and at farrowing. These results match those reported by Bourne (1969) and Klobasa *et al.* (1987), who reported that colostrum Ig concentration at birth (in mg/mL) decreased from 95.6 at the beginning of lactation to 64.8 after 6 h, and to 32.1 after 12 h. Based on the literature, at present ELISA is the analytical method most commonly used to measure swine colostrum IgG concentration. However this analysis requires specific laboratory equipment, the kit cost is very high, it needs expertise to run the test and the results are not immediate. The RID test, in comparison with ELISA, requires less experience but it remains a laboratory analysis, which is expensive and time consuming. These

results indicate that the Brix refractometer can be a useful on-farm tool and can cheaply generate large scale data, with important implications for improved selection of sows with better maternal characteristics.

Future research will investigate whether it could be also possible to assess failed transfer of passive immunity by measuring the piglet serum IgG with the refractometer, as has been done in calves (Chigerwe and Hagey, 2014) and cows (Morrill *et al.*, 2013).

Conclusion

In accordance with the hypothesis for the work, the results indicate that the Brix refractometer can be used as an on-farm method to assess IgG concentration in swine colostrum, with a positive correlation between Brix percentage and the gold standard RID values. The Brix refractometer results in this investigation demonstrate that the method is highly repeatable and that storage temperature and duration do not affect the IgG assessment. Colostrum concentration changed by four hours after the birth of the first piglet, and so sampling time needs to be taken into account as IgG concentration decreased significantly. The Brix refractometer is durable and affordable, expertise is not necessary and the calibration process is simple, making it a very practical farm tool. Because sow colostrum IgG concentration is not yet well estimated, the refractometer can be used to investigate the IgG variation in colostrum produced under field conditions, reducing cost and time, and facilitating research requiring a large sample size. Moreover, incorporating the use of a refractometer into colostrum management on farm, will allow farmers to feed weak piglets with good quality colostrum, improving welfare and economy by reducing piglet mortality.

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References

- Ariza-Nieto C, Bandrick M, Baidoo SK, Anil L, Molitor TW and Hathaway MR 2011. Effect of dietary supplementation of oregano essential oils to sows on colostrum and milk composition, growth pattern and immune status of suckling pigs. *Journal of Animal Science* 89, 1079-1089.
- Bartier AL, Windeyer MC and Doepel L 2015. Evaluation of on-farm tools for colostrum quality measurement. *Journal of Dairy Science* 98, 1878-1884.
- Bielmann V, Gillan J, Perkins NR, Skidmore AL, Godden S and Leslie KE 2010. An evaluation of Brix refractometry instruments for measurement of colostrum quality in dairy cattle. *Journal of Dairy Science* 93, 3713-3721.
- Bland IM, Rooke JA, Bland VC, Sinclair AG and Edwards SA 2003. Appearance of immunoglobulin G in the plasma of piglets following intake of colostrum, with or without a delay in sucking. *Animal Science* 77, 277-286.
- Boulot S 2009. Hyperprolific Sow, Physiological or Pathological? *Reproduction in Domestic Animals* 44, 66-66.
- Bourne FJ 1969. Antibody transfer and colostral whey proteins in the pig. *The Veterinary record* 84, 607-608.
- Cabrera RA, Lin X, Campbell JM, Moeser AJ and Odle J 2012. Influence of birth order, birth weight, colostrum and serum immunoglobulin G on neonatal piglet survival. *Journal of animal science and biotechnology* 3, 42-42.
- Cash RSG 1999. Colostral quality determined by refractometry. *Equine Veterinary Education* 11, 36-38.

Chavatte P, Clement F, Cash R and F. G 1998. Field determination of colostrum quality by using a novel, practical method. *American Association of Equine Practitioners* 44, 206-209.

Chigerwe M and Hagey JV 2014. Refractometer assessment of colostrum and serum IgG and milk total solids concentrations in dairy cattle. *Bmc Veterinary Research* 10, 6.

Chigerwe M, Tyler JW, Middleton JR, Spain JN, Dill JS and Steevens BJ 2008. Comparison of four methods to assess colostrum IgG concentration in dairy cows. *Javma-Journal of the American Veterinary Medical Association* 233, 761-766.

Csapo J, Martin TG, CsapoKiss ZS and Hazas Z 1996. Protein, fats, vitamin and mineral concentrations in porcine colostrum and milk from parturition to 60 days. *International Dairy Journal* 6, 881-902.

Csapo J, Stefler J, Martin TG, Makray S and Csapokiss Z 1995. Composition of mares colostrum and milk - fat-content, fatty-acid composition and vitamin content. *International Dairy Journal* 5, 393-402.

Curtis SE 1970. Environmental-thermoregulatory interactions and neonatal piglet survival. *Journal of Animal Science* 31, 576-&.

Devillers N, van Milgen J, Prunier A and Le Dividich J 2004. Estimation of colostrum intake in the neonatal pig. *Animal Science* 78, 305-313.

Foisnet A, Farmer C, David C and Quesnel H 2011. Farrowing induction induces transient alterations in prolactin concentrations and colostrum composition in primiparous sows. *Journal of Animal Science* 89, 3048-3059.

Fraser D 1984. Some factors influencing the availability of colostrum to piglets. *Animal Production* 39, 115-123.

Gapper LW, Copestake DEJ, Otter DE and Indyk HE 2007. Analysis of bovine immunoglobulin G in milk, colostrum and dietary supplements: a review. *Analytical and Bioanalytical Chemistry* 389, 93-109.

Harker DB 1978. Simple estimation of immunoglobulin content of ewe colostrum. *Veterinary Record* 103, 8-9.

Inoue T 1981a. Possible factors influencing immunoglobulin-a concentration in swine colostrum. *American Journal of Veterinary Research* 42, 533-536.

Inoue T 1981b. Possible factors influencing the immunoglobulin-m concentration in swine colostrum. *American Journal of Veterinary Research* 42, 1429-1432.

Kehoe SI, Jayarao BM, Heinrichs AJ 2007. A survey of bovine colostrum composition and colostrum management practises on pennsylvania dairy farms. *Journal of Dairy Science* 90, 4108-4116.

Klobasa F, Werhahn E and Butler JE 1987. Composition of sow milk during lactation. *Journal of Animal Science* 64, 1458-1466.

Machadoneto R, Graves CN and Curtis SE 1987. Immunoglobulins in piglets from sows heat-stressed prepartum. *Journal of Animal Science* 65, 445-455.

Morin DE, Constable PD, Maunsell FP and McCoy GC 2001. Factors associated with
 colostral specific gravity in dairy cows. *Journal of Dairy Science* 84, 937-943.

Morrill KM, Robertson KE, Spring MM, Robinson AL and Tyler HD 2015. Validating a
 refractometer to evaluate immunoglobulin G concentration in Jersey colostrum and the effect
 of multiple freeze-thaw cycles on evaluating colostrum quality. *Journal of Dairy Science* 98,
 595-601.

Morrill KM, Polo J, Lago A, Campbell J, Quigley J and Tyler H 2013. Estimate of serum
 immunoglobulin G concentration using refractometry with or without caprylic acid
 fractionation. *Journal of Dairy Science* 96, 4535-4541.

Morrill KM, Conrad E, Polo J, Lago A, Campbell J, Quigley J and Tyler H 2012. Estimate of
 colostral immunoglobulin G concentration using refractometry without or with caprylic acid
 fractionation. *Journal of Dairy Science* 95, 3987-3996.

Porter P 1969. Transfer of immunoglobulins IgG, IgA and IgM to lacteal secretions in the
 parturient sow and their absorption by the neonatal piglet. *Biochimica et Biophysica Acta*
 181, 381-392.

Quesnel H 2011. Colostrum production by sows: variability of colostrum yield and
 immunoglobulin G concentrations. *Animal* 5, 1546-1553.

Quesnel H, Farmer C and Devillers N 2012. Colostrum intake: Influence on piglet
 performance and factors of variation. *Livestock Science* 146, 105-114.

Quigley JD, Lago A, Chapman C, Erickson P and Polo J 2013. Evaluation of the Brix
 refractometer to estimate immunoglobulin G concentration in bovine colostrum. *Journal of*
Dairy Science 96, 1148-1155.

Wolak ME, Fairbairn DJ and Paulsen YR 2012. Guidelines for estimating repeatability.
Methods in Ecology and Evolution 3, 129-137.

Table 1. Descriptive statistics of 124 colostrum samples measured at different sampling times in relation to farrowing using a Brix refractometer, and of a subset of 42 samples measured by radial immunodiffusion (RID).

<i>Value</i>	<i>N</i>	<i>Mean</i>	<i>SD</i>	<i>Minimum</i>	<i>Maximum</i>
Brix fresh (%)	124	24.1	2.65	18.3	33.0
Sampling time:					
Before^a (h)	38	-03:17:48	02:36:25	-00:11:00	-09:00:00
During^b (h)	49	00:56:20	00:59:22	00:00:00	03:22:00
After^c (h)	37	09:49:26	03:34:07	04:02:00	14:30:00
RID (mg/mL)	42	22.4	5.43	13.3	35.0

^a Sampling time before farrowing. ^b Sampling time during farrowing. ^c Sampling time after farrowing.

Table 2. *Immunoglobulin concentration measured via Refractometer (Brix %, N = 124), and radial immunodiffusion (RID, N = 42) (mg/mL) at different sampling times.*

<i>Sampling time (h)</i>	Brix (%)					RID (mg/mL)				
	<i>N</i>	<i>Mean</i>	<i>SD</i>	<i>Min</i>	<i>Max</i>	<i>N</i>	<i>Mean</i>	<i>SD</i>	<i>Min</i>	<i>Max</i>
Before ¹	38	24.8 ^a	3.06	18.3	31.3	13	22.8 ^a	4.33	14.6	28.0
During ²	49	24.6 ^a	2.18	20.3	33.0	18	23.7 ^a	5.35	15.2	35.1
After ³	37	22.6 ^b	2.08	18.3	26.2	11	20.2 ^b	4.43	13.3	26.7
sem		0.41					0.07			
P		0.0008					0.0001			

Mean, standard deviation and range.

^{a,b} Within a column, means without a common letter differ (P < 0.05).

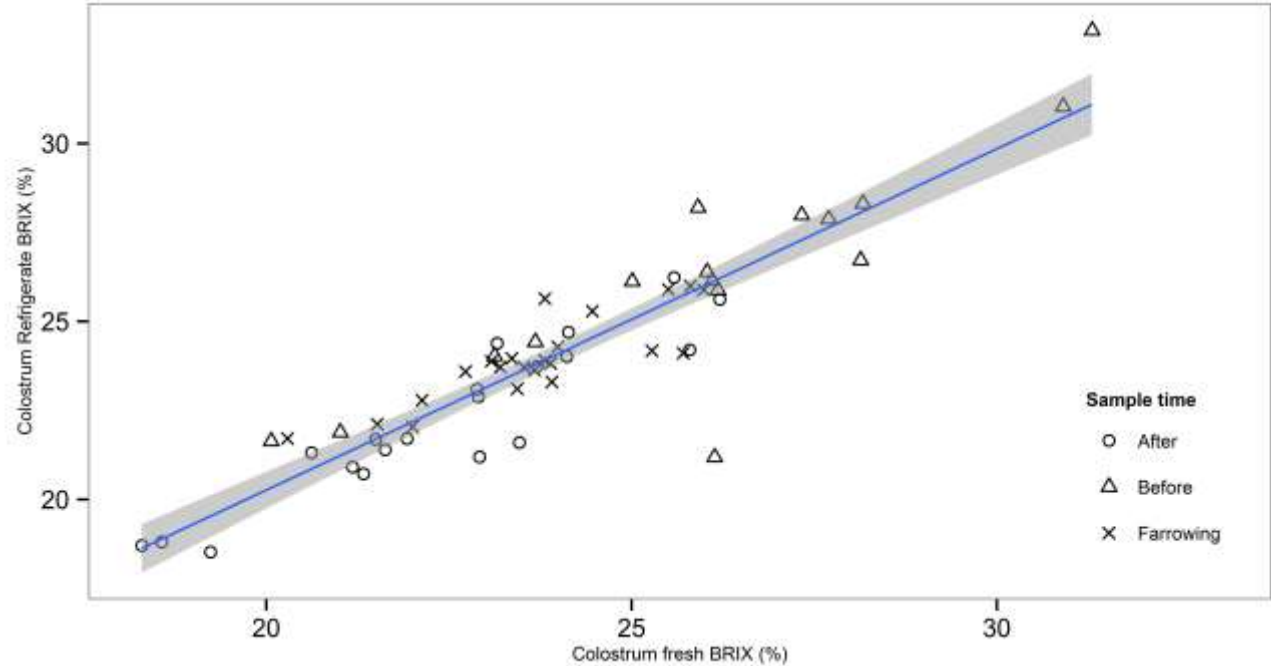
¹ Before farrowing (-9h to 0h). ² During farrowing (0h-4h). ³ After farrowing (4h-14h).

List of figure captions

Figure 1. Regression line of Brix refractometer (%) values of fresh and refrigerated samples taken at different sampling times (samptime) ($r = 0.86$, $P < 0.05$), also illustrating the effects of different sampling time ($F_{2,110} =$; $P < 0.001$) (After= after farrowing, Before= before farrowing, Farrowing= during farrowing). The grey area represents the confidence interval of the mean.

Figure 2. Regression plot between radial immunodiffusion (RID) immunoglobulin G (IgG) concentration and Refractometer Brix (%) of 42 colostrum samples (Regression equation: $RID = 1.01(\pm 0.2) \text{ Brix} + 1.94(\pm 5.6)$; $R^2 = 0.31$, $P < 0.001$). The grey area represents the confidence interval of the mean.

459 **Fig 1**

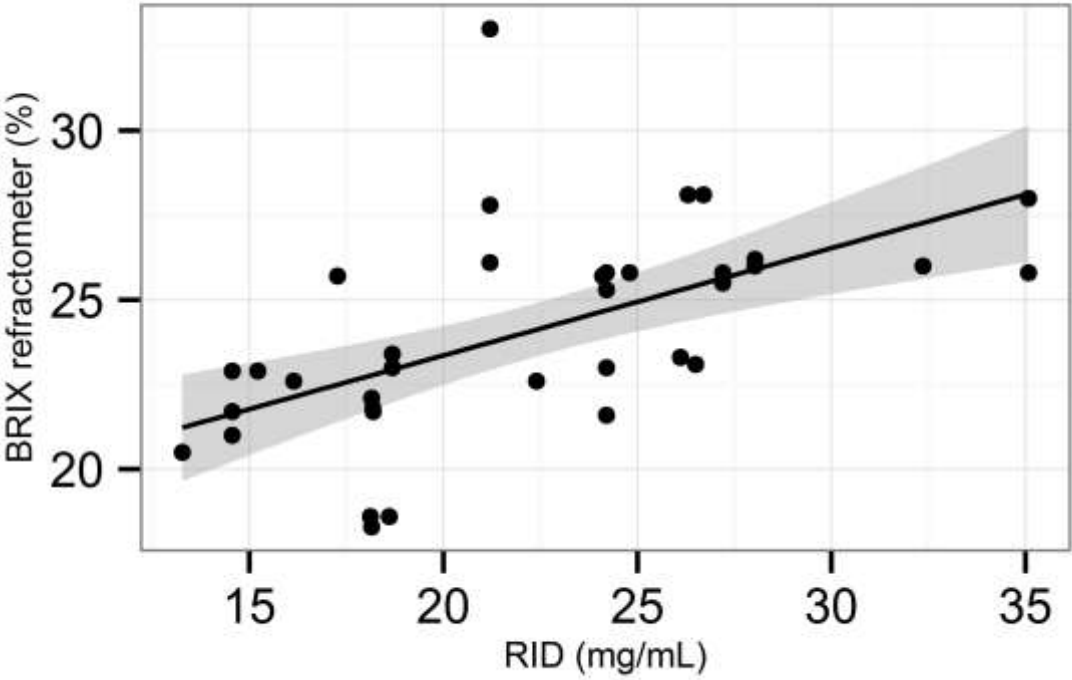


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462 **Fig 2**

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